

AGRICULTURE

PCR-Based Identification of Streptomycin Resistance in *Salmonella* Serovars Using a Novel Primer Set

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Streptomycin has been used to treat bubonic plague and tularemia and, as a second line of antibiotic drug of choice, to treat tuberculosis. Streptomycin is also used as a pesticide to control bacteria, fungi, and algae in zoonotic and plant diseases. Extensive use of antibiotics has resulted in the emergence of antibiotic resistance in bacterial pathogens. Sheng Chen and colleagues (2004) have shown that 73 of 133 *Salmonella* isolates obtained from retail meats in the U.S. and China were resistant to at least one antimicrobial agent, and 61% of them were resistant to streptomycin. To understand antimicrobial resistance in *Salmonella*, it is important to develop molecular and biophysical tools to study streptomycin resistance.

In this study, we have designed and used novel primers specific for *aadA* gene coding for aminoglycoside adenylyltransferase (required for streptomycin resistance) in the top 20 *Salmonella* serovars of human foodborne outbreak origins. Primers (aadA-F: 5'-TCCACCTTCCAGGGAAATGC-3'; aadA-R: 5'-GGGTATACCAGATACGCGCC-3') were designed by using the Primer-BLAST program ([http://www.ncbi.nlm.](http://www.ncbi.nlm.nih.gov/tools/primer-blast/)

[nih.gov/tools/primer-blast/](http://www.ncbi.nlm.nih.gov/tools/primer-blast/)). *Salmonella* cultures were grown for three hours to extract total DNA by boiling method. PCR amplicon of size 287 bp was obtained from *Salmonella* serovars, while non-*Salmonella* serovars did not yield any amplification. In the bacterial growth inhibition assay, eleven *Salmonella* serovars exhibited significant growth ($A_{595} > 0.4$) in the presence of streptomycin (100 $\mu\text{g/ml}$) cultured in a 96-well microtiter plate (Microtest U-Bottom, BD), indicating strong antibiotic resistance. The nine remaining serovars did not show considerable growth ($A_{595} < 0.4$) as monitored by a spectrophotometer, indicating their sensitivity to streptomycin. All non-*Salmonella* cultures tested were also sensitive to streptomycin. This study lays the foundation for a future objective, to examine expression of aminoglycoside adenylyltransferase in the *Salmonella* serovars that carried the *aadA* gene but failed to grow in the presence of streptomycin.

Research advisor Arun Bhunia and postgraduate Atul K. Singh explain, "Antibiotic resistance in human pathogens poses a serious public health risk. Developing molecular methods, including PCR assays targeting resistance genes, will help scientists understand the genotypic and phenotypic relationships in antibiotic resistance. Exposure of students, especially those in the early stage of their careers, to such global challenges helps develop skills required for investigative solutions."

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